stem cells into endothelial cells. The stem cells were derived from bone marrow by CD34 selection, and were stimulated with either m-CSF or PTN alone or a combination of m-CSF and PTN or no treatment for 7 days. Real-time PCR analysis showed that the m-CSF and PTN combination markedly increased endothelial cell marker expression and decreased monocyte marker (CD68 and *c-fms*) expression in this stem cell population. When induced with PTN alone, the stem cells exhibited slightly increasing expression of endothelial markers. These experiments define a previously unrecognized novel mechanism leading to angiogenesis in cancer patients: the transdifferentiation of monocytes into endothelial cells by a factor highly produced by tumor cells. They also suggest a potential new specific target to inhibit angiogenesis—pleiotrophin—which may have profound clinical implications.

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BONE MARROW STEM CELL TRANSPLANTATION IN NEURODEGENERATIVE AND CEREBROVASCULAR DISEASE: PRELIMINARY REPORT

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Stem cells with self-renewal and multilineage potential are considered good candidates for cell replacement of damaged nervous tissue in cerebrovascular and neurodegenerative disease. Most of the knowledge about the role of stem cells in the progression of these diseases have been based on experimental evidence. Until now, there has been no human study showing the effectiveness of stem cell transplantation in neurologic disease. In this study we attempted to test whether bone marrow stem cells can improve the functional, radiologic, and metabolic outcomes in patients with vascular dementia and ischemic stroke with intra-arterial injection.

Seven patients, (3 female and 4 male; mean age ± SD, 68.23 ± 11.2 years) were included in the study. Patients were selected from among chronic poststroke and/or vascular dementia patients who did not respond to classical therapy. Barthel, NIH, and MMSE scales were used to record mental and motor status, and MR and SPECT were used to record radiologic and metabolic status before and 30, 60 and 120 days after the procedure. After the patients were given neurologic examinations, their bone marrow was aspirated, cultured in Teflon bags, and proliferated during 24 hours. Cultured bone marrow was given to patients intra-arterially and angiographically under sterile conditions.

Functionally and mentally, all patients showed improvement in Barthel, NIH, and MMSE scales 60 days after the procedure, but the differences were not statistically significant (first Barthel, 9.85 ± 2.67, last Barthel, 11.28 ± 2.69, [$P = .32$]; first NIH, 9.00 ± 2.63, last NIH, 7.42 ± 2.19 [$P = .16$]; first MMSE, 10.71 ± 3.73, last MMSE, 17.00 ±4.69 [$P = .63$]).

In terms of radiology, volumetric improvement on MR was seen after the procedure (Cavalier volumetric method; $P = .047$). On SPECT, cerebral metabolism was observed to increase in 3 patients quantatively. Finally, we observed a trend of clinical improvement in our patients. But our study has some limitations, such as a small number of patients. Nonetheless, in light of our first results, we can say that otology bone marrow stem cell transplantation may provide new hope in the treatment of neurodegenerative disease.

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BIOLUMINESCENT TRACKING OF CANDIDATE LEUKEMIC STEM CELL ENGRAFTMENT IN IMMUNOCOMPROMISED MICE

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Background: We investigated whether leukemic stem cells (LSCs) involved in CML progression use the beta-catenin pathway for self-renewal and also whether inhibition of beta-catenin with axin prevents engraftment of leukemic stem cells in an immunocompromised mouse model using bioluminescent tracking. **Methods:** We used fluorescence-activated cell sorting (FACS) to isolate hematopoietic stem cells (HSCs), common myeloid progenitors (CMPs), granulocyte/macrophage progenitors (GMPs), and megakaryocyte/erythroid progenitors (MEPs) from normal marrow, cord blood, or advanced-phase CML. In vitro replating assays were used to identify self-renewing cells as candidate leukemic stem cells, and the dependence of self-renewal on beta-catenin activation was tested by lentiviral transduction of hematopoietic progenitors with axin, an inhibitor of the beta-catenin pathway. CML HSCs, CMPs, GMPs, or CD34+38+ cells were transduced with a lentiviral luciferase GFP vector with or without axin, transplanted intrahepatically into newborn T-, B-, and NK-deficient mice and monitored for engraftment via weekly bioluminescent imaging as well as tail bleeding to detect GFP-positive cells. **Results:** When compared with normal marrow, the GMP pool from patients in blast crisis and imatinib-resistant CML was expanded and had elevated levels of nuclear beta-catenin. Unlike normal GMPs, CML GMPs formed self-renewing replatable myeloid colonies, and in vitro self-renewal capacity was reduced by enforced expression of axin. Although CML HSC showed evidence of early engraftment, committed progenitors, such as GMP, expanded more readily in vivo. and their growth was inhibited by pretreatment with axin. **Conclusions:** Activation of beta-catenin in CML granulocyte/macrophage progenitors likely enhances their self-renewal and leukemic activity. Specific beta-catenin inhibitors such as axin block this aberrant self-renewal activity in vitro and in vivo. Finally, the use of bioluminescent imaging of leukemic/cancer stem cell engraftment in vivo using a newborn immunocompromised mouse model provides a robust tool for assessing response of cancer stem cells to molecularly targeted therapy.

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EFFECTS AND KINETICS OF ANTI–C-KIT MONOCONAL ANTIBODY ACK-2 ON HEMATOPOIESIS AND HEMATOPOIETIC PROGENITORS AND ITS ABILITY TO CONDITION FOR BONE MARROW TRANSPLANTATION

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Background: The stem cell receptor factor receptor c-Kit is expressed on hematopoeitic stem cell (HSC) and progenitor cell populations. ACK-2 is an anti-mouse c-kit mAb that has been shown to antagonize the function of c-kit and deplete the bone marrow of treated mice. We wished to characterize the effect and kinetics of ACK-2 on the peripheral blood cell counts and marrow HSC and progenitor populations. We also tested the hypothesis that treatment with ACK-2 may serve as a novel means of nonmyeloablative conditioning for HSC transplantation. **Methods:** Adult C57/BL mice were injected either intravenously or intraperitonealy (IP) with 1 mg of ACK2 mAb on days 0, 2, and 4. Peripheral blood cell counts, including WBC differentials, were followed over time in recipient mice. Peripheral blood and bone marrow was analyzed at day 7, and marrow was analyzed for fraction and proportions of HSC, common myeloid progenitors (CMPs), granulocyte macrophage progenitors (GMPs), and macrophage erythrocyte progenitors (MEPs). A subset of Ly5.1 mice that had received ACK2 mAb on days 0, 2, and 4 received a bone marrow transplantation on day 7 with 1×10^6 bone marrow cells from a congenic Ly5.2 donor. **Results:** Both IV and IP administration of ACK-2 resulted in rapid development of anemia, neutropenia, and thrombocytopenia (see the table for results). Analysis of peripheral blood revealed a decrease in Mac-1+ cells from 12.2% of circulating WBCs in untreated controls to 3.1% at day 7, whereas the proportion of B220+ B cells increased from mean of 49.7% in controls to 72.7%. The fraction of circulating T cells decreased from 24.5% to 11.1% at day 7. The bone marrow fraction of KTLS HSC decreased from 0.07% of total marrow cells in controls to 0.003% in ACK-2–treated mice. The marrow fractions of CMP and GMP all decreased in treated mice, with a proportional drop in CMP progenitors from 12% in untreated mice to 4.7% in treated mice. ACK-2–treated mice transplanted with Ly5.2 congenic marrow and analyzed at D+21 showed no evidence of donor-derived cells in the peripheral blood, compared with 83% Ly5.2 donor-derived cells in control mice receiving 9 Gy of radiation. **Coclusions:** The anti–c-kit mAb ACK-2 rapidly induces anemia, neutropenia, and thrombocytopenia with correlating decreases in the numbers of marrow HSC and other progenitor populations. Despite the ablative action of the ACK-2 on marrow populations, it does not appear to enable engraftment of syngeneic bone marrow.

Table 1. *Effect of Anti c-Kit mAb on Peripheral Blood Cell Counts*

	Day 0 Control	Day 2	Day 4	Day 7	Day 11
WBC	12.1	6.6	4.2	2.6	9.5
Hgb	15.1	14.5	13.2	6.2	5.3
ANC	796	180	32	26	320
Platelet Count	1107	1182	1098	763	220

Mice injected with 1 mg ACK-2 intravenously on Days 0, 2 & 4.

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EPITHELIAL CHIMERISM IN THE ORAL MUCOSA AFTER HUMAN HEMATOPOIETIC CELL TRANSPLANTATION

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Several study groups have reported on a nonhematopoietic chimerism following allogeneic hematopoietic cell transplantation (aHCT) both in animals and humans. We recently investigated this phenomenon in humans and described the pitfalls of identification of epithelial chimerism (Spyridonidis et al, Am J Pathol 164:1147–1155, 2004). The purpose of the present study was to examine epithelial chimerism in single cells isolated from the oral mucosa. Buccal scrapings obtained from 13 female patients who underwent a sex-mismatched aHCT were employed to prepare cytospins. The examination was performed 75–1964 days after aHSCT. At this time point, no patient had signs of mucositis or oral GvHD. Cytospin preparations were examined with a combination of FISH for the Y or the XY chromosome; immunofluorescent stain for the epithelial-specific marker cytokeratin (CK), for CD45, and Drag cell nuclei stain; with APAAP immunocytochemistry using either CD15-, CD45-, CD68-, or CD3-specific antibodies; and with hematoxylin and eosin (HE) staining. Evaluation of epithelial chimerism was performed with laser scanning confocal microscopy from examiners who were not aware of the patients' present or past medical history. CD45+ or CD3+ cells were found in 4 patients with a frequency of < 1%. No CD68+ or CD15+ positive macrophages were detected. In the FISH-Y combined stains, we detected Y+/CK+/CD45− cells in 12 of 13 patients (92%), with a mean of 1.8% Y+/CK+/CD45− cells per 200 total cells analyzed (range, 0.5%-7.3%). The identified Y+/CK+/CD45− cells were counterstained with HE and revealed an epithelial morphology. Screening of 5 patients with XY stain demonstrated all Y+ cells of them having only 1 X chromosome, making fusion as the underlying mechanism unlikely. We retrospectively reviewed the transplantation documents of every patient and found a significant correlation (P value = .0028) between the severity of mucositis in the early posttransplantation period (up to day +30) and the degree of epithelial chimerism found at later time points (days +75- +1964), where no signs of mucositis were present. We conclude that epithelial chimerism of the oral mucosa is a real phenomenon after aHSCT. Further molecular investigation on a single-nucleus level and analysis of greater number of transplanted patients are needed to understand the underlying mechanisms responsible for generation of the donor derived epithelial cells. Tissue injury seems to play a role in these mechanisms.

SUPPORTIVE CARE

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PEGFILGRASTIM VERSUS FILGRASTIM TO ACCELERATE HEMATOPOIETIC RECOVERY AFTER HIGH-DOSE MELPHALAN AND AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (ASCT) FOR MULTIPLE MYELOMA

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High-dose melphalan followed by ASCT is a common component of the early treatment for multiple myeloma. Daily subcutaneous injections of filgrastim (Neupogen) at 5 μg/kg/day until ANC > 500/μL are routinely administered at our center from day +4 following ASCT, to accelerate hematopoietic recovery and lessen neutropenic complications. Pegfilgrastim (Neulasta) as a single 6-mg fixed dose via subcutaneous injection has been shown to have similar efficacy and ease of use as filgrastim in the nontransplantation setting, but little data are available in the transplantation setting. We began using pegfilgrastim on day +1 following ASCT for patients with multiple myeloma and performed a retrospective cohort study comparing those who received filgrastim (the filgrastim group [FG]; n = 6) with those who received pegfilgrastim (the pegfilgrastim group [PG]; n = 11). Transplantations occurred between July 2002 and January 2004 and included all patients transplanted for myeloma in that period for whom sufficient data were available. All patients had peripheral stem cells harvested after cytoxan/filgrastim mobilization. Main outcome measures were days from stem cell infusion to WBC nadir, days to ANC > 500/μL, and days to ANC > 1000/μL. Subjects were excluded if CBCs were drawn less often than every 4 days. There were no significant differences between the FG and the PG with respect to the following variables: age, gender, hemoglobin, creatinine, calcium, albumin, beta-2 microglobulin, number of prior lines of therapy, and number of CD34+ cells infused. After transplantation, the median number of days to WBC nadir was 7 (range, 5–9) in the FG and 6 (range, 5–8) in the PG (P = .31). However, median number of days to ANC > 500/μL was 11.5 (range, 11–17) in the FG and 10 (range, 9–12) for the PG (P = .02). Similarly, median number of days to ANC > 1000/μL was 12 (range, 11–17) for the FG and 11 (range, 10–13) for the PG (P = .03). Five of 6 patients in the FG had neutropenic fever after transplantation, compared with 5 of 11 patients in the PG (P = .30). Currently, no significant differences in infection or relapse rates between the groups was noted, and there were no deaths in either group. In this retrospective cohort study, pegfilgrastim was safe and at least equivalent to filgrastim for accelerating hematopoiesis after ASCT for multiple myeloma. Furthermore, there was no significant difference in the incidence of neutropenic fever, infection, and survival, suggesting a similar clinical utility.

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VANGANCICLOVIR PROPHYLAXIS FOR THE PREVENTION OF CYTOMEGALOVIRUS REACTIVATION AND DISEASE IN ALLOGENEIC STEM CELL TRANSPLANTATION RECIPIENTS

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Background: CMV infection continues to be an important cause of morbidity and mortality after allogeneic stem cell transplantation. Valganciclovir (VGC), the valine ester of ganciclovir, has excellent oral bioavailability and has the potential to replace intravenous ganciclovir in many situations, namely CMV prophylaxis or preemptive therapy after allogeneic transplantation. **Methods:** From October 2002 to April 2004, 34 patients who were either CMV seropositive or had a seropositive donor were enrolled in prospective trial of intravenous (IV) ganciclovir at 5 mg/kg twice a day for 1 week, followed by oral VGC 900 mg twice a day for a total of 180 days. IV ganciclovir was started at time of engraftment. Dose adjustments of VCG were made according to renal function, and growth factors were allowed in the event of neutropenia. Study endpoints included incidence of CMV reactivation and disease during the first 180 days after transplantation. For the study, viremia was defined as a positive CMV blood culture by shell vial or conventional culture. A positive antigenemia assay in patients with severe (grade III-IV) GVHD was defined as 1 positive cell on either of 2 duplicate slides. For patients with no or mild GVHD (grade I-II), a positive antigenemia assay was defined as 2 positive cells/slide. **Results:** Thirty-four patients were enrolled. Thirteen patients were not able to proceed after consenting due to positive antigenemia prior to starting study (3 patients), financial reasons (3 patients), severe gut GVHD unabling oral intake (5 patients), or death (2 patients). Of those who received VGC, the median age